Streptococcus pyogenes YtgP (Spy_0390) Complements *Escherichia coli* Strains Depleted of the Putative Peptidoglycan Flippase MurJ[▽]

Most bacteria possess peptidoglycan (PG), an extracytoplasmic polymer that protects them from osmotic lysis and that serves as an anchor for cellular components, including virulence factors (10). PG biogenesis is the target of many antibiotics, but increasing resistance is rendering many such antibiotics ineffective (6). Recently, MurJ (formerly MviN) has been shown essential for PG biogenesis in *Escherichia coli* and has been proposed to function as the flippase that translocates the PG precursor lipid II across the cytoplasmic membrane (3, 7). Because of the clear potential of MurJ as a novel antibiotic target, we need to understand both how MurJ functions and how conserved MurJ is in bacteria.

A BLASTP 2.2.20+ search (1, 2) revealed that MurJ is highly conserved among gram-negative bacteria (*Proteobacteria*) but not among 21 genomes representative of the gram-positive *Firmicutes* (including *Bacillus*, *Listeria*, *Staphylococcus*, *Enterococcus*, and *Streptococcus*), since it found only one protein with an E value of <0.01, YtgP (locus tag BSU30050) of *Bacillus subtilis* subsp. strain 168. Failure to identify the putative PG flippase in key gram-positive pathogens begged the question of whether these organisms have an ortholog that does not share a high degree of sequence homology with *E. coli* MurJ or whether they flip lipid II through a different mechanism. The first possibility seemed more likely, since BLASTP revealed that *Bacillus* YtgP is highly conserved among *Firmicutes*.

To investigate whether YtgP is the *E. coli* MurJ ortholog, I tested whether YtgP complements MurJ-depleted *E. coli* cells. The algorithm ParAlign, which is more sensitive than BLASTP(8), showed that there are several YtgP paralogs in *B. subtilis* (EpsK, SpoVB, YfkQ, and YabM); therefore, I chose the YtgP from an organism encoding a single ortholog, namely, Spy_0390 (GenBank accession no. AAK33429.1) from *Streptococcus pyogenes*. First, the Spy_0390 gene from *S. pyogenes* M6 strain HSC5 was amplified using PCR primers Sp5ORF0390 (5'-GCCTCGACAGAAAAAACAATGACACAAGAAGATTG) and Sp3ORF0390 (5'-CCATGCCAGCCTAATTTCTGACGAAGGCGACT), and the blunt-ended product was introduced into StuI-digested pCA24N(-gfp) (4) to yield pSpyMurJ1, which produces Spy_0390 under the control of an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible promoter as a hybrid protein with an N-terminal MRGS-His₆-TDP ALRA peptide fused to its second amino acid and a C-terminal GLCGR peptide fused to its last amino acid. For controls, ASKA collection (4) plasmids pCA24N(-gfp) and its derivative carrying the *E. coli* murJ were used and renamed pCA24Not and pEcMurJ1, respectively. Chloramphenicol (20 μg/ml) was used for plasmid selection.

Vectors pCA24Not, pEcMurJ1, and pSpyMurJ1 were introduced into *E. coli* strain NR1152, which produces MurJ in the presence of arabinose but lyses when grown in the absence of arabinose because of the depletion of MurJ (7). To quantify complementation, efficiency of plating values of overnight cultures were determined by plating serial dilutions on Luria-Bertani (LB) medium alone and LB medium containing either arabinose or IPTG (Table 1). An efficiency of plating value of 1 was assigned to *E. coli* NR1152(pCA24Not)

TABLE 1. Efficiency of plating values of *E. coli* strain NR1152 carrying pCA24Not-derived plasmids on various media

Plasmid	Efficiency of plating value ^a of <i>E. coli</i> NR1152 carrying plasmid on the following medium:		
	LB medium with 0.07% arabinose	LB medium alone	LB medium with 30 μM IPTG
pCA24NotI	1	<10 ⁻⁴	<10 ⁻⁴
pEcMurJ1	0.5	0.8	1
pSpyMurJ1	0.8	<2 × 10 ⁻³	0.8

^a All values were calculated by dividing the number of colonies obtained for each strain under each condition by the number of colonies that *E. coli* NR1152(pCA24NotI) yielded in the presence of arabinose. These data are representative of at least three independent experiments.

plated on LB containing arabinose. As expected, strain NR1152 (pCA24Not) only grew with arabinose. *E. coli* NR1152 (pEcMurJ1) grew with arabinose or IPTG; it also grew on LB owing to leaky expression of *E. coli* murJ from the plasmid (4). *E. coli* NR1152(pSpyMurJ1) grew well with arabinose and not without arabinose, but IPTG fully restored growth, albeit it yielded smaller colonies than NR1152(pEcMurJ1) did. The lower rate of growth of *E. coli* NR1152(pSpyMurJ1) compared to NR1152(pEcMurJ1) is likely caused by lower expression of the streptococcal protein owing to differences in codon usage between the two bacteria, since the streptococcal sequence includes several codons rarely used in *E. coli*. Moreover, passage of *E. coli* NR1152(pSpyMurJ1) through medium containing high levels of IPTG selected for mutations in the IPTG-inducible promoter region of pSpyMurJ1 that restored wild-type colony size. Restoration of wild-type growth was proven to be linked to these promoter mutations in the plasmid; thus, once appropriate levels of Spy_0390 gene expression are achieved, Spy_0390 protein complements fully.

These results indicate that Spy_0390 and *E. coli* MurJ are functional homologs, so they suggest that YtgP orthologs, which are widely spread among gram-positive bacteria, likely function as PG lipid II flippases. As expected from such function, YtgP is essential in *S. pneumoniae* and *Staphylococcus aureus* (9, 11). Notably, YtgP is not essential in *B. subtilis* probably because of redundancy among the aforementioned paralogs (5). The cross-phylum complementation reported here has two implications important for the development of MurJ inhibitors. First, because lipid II in *E. coli* differs from that of *S. pyogenes* (10), MurJ likely recognizes features in lipid II that are common in both phyla, namely, the undecaprenylpyrophosphoryl disaccharide. Second, it might be possible to find inhibitors of these putative lipid II flippases that will be broad-spectrum antibiotics.

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